Determinants of Carotid Artery Wall Thickening in Young Patients with Type 1 Diabetes Mellitus

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To investigate associations between early atherosclerosis and possible risk factors for it in young patients with established Type 1 diabetes mellitus (DM), we measured the combined intima-media thickness (IMT) of the common carotid arteries with high resolution ultrasound in 310 young patients (age $\leq$ 40 years, mean 27.9 $\pm$ 6.5) with a diabetes duration $\geq$ 2 years, and in two control groups of similar age (control 1:40 healthy subjects, control 2: 40 Type 1 DM recently diagnosed patients). Albumin excretion rate and lipids (total cholesterol and triglycerides) were measured and retinopathy and hypertension (systolic blood pressure $>$ 140 or diastolic blood pressure $>$ 90 mmHg) sought in the patients. Mean maximum IMT was 0.52 $\pm$ 0.06 mm in control group 1 and 0.50 $\pm$ 0.05 mm in control group 2 with a mean difference of 0.02 mm (95% CI: −0.01, 0.04). The more established Type 1 DM patients had a significantly greater IMT (0.57 $\pm$ 0.13 mm, $p < 0.001$) than both control groups. In a subgroup analysis, patients with microvascular diabetic complications ($n = 99$) had a significantly greater IMT (0.63 $\pm$ 0.17 vs 0.55 $\pm$ 0.10 mm, $p < 0.001$) than those without ($n = 211$). In a multiple linear regression analysis with a significance level of $\leq$ 0.10, the carotid artery IMT of our established diabetic patients was related to age, male gender, triglycerides and nephropathy, suggesting the latter as the main diabetes-specific risk for intima-media thickening in young Type 1 DM patients.

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Introduction

With high resolution ultrasound it is possible to measure the wall thickness of larger arteries as the combined intima-media thickness (IMT). Patients with cardiovascular risk factors, especially hypercholesterolaemia, have a marked thickening of the carotid artery wall,[1–4] and there are also associations between IMT and smoking, systolic hypertension, coronary heart disease, and Type 2 diabetes mellitus (DM).[2,5–12]

A thickening of the carotid artery wall is regarded as an early stage of atherosclerosis. The development of plaques indicates an advanced stage.[1,6,7,9,13–17] The atherosclerosis of the carotid artery shows a progression similar to that in other arteries, especially the coronary arteries.[6,7,15] So the carotid artery IMT can be taken to reflect the extent of general and coronary atherosclerosis.[2,3,6–10,13]

There are few studies of the IMT in diabetic patients and these are mostly in Type 2 disease.[18–22] However, carotid artery wall thickening may occur also in patients with Type 1 DM.[23–26] In an earlier study,[27] we found changes of the carotid artery wall (increased IMT and/or plaques) in about 20 % of young patients with Type 1 DM, mostly in those with microvascular complications.

The conditions which lead to early atherosclerosis in young patients with Type 1 DM are not known. Besides the classical cardiovascular risk factors, albuminuria seems to play a major role.[28,29] It is also not known whether Type 1 DM alone is a risk for the development of premature atherosclerosis. The aim of this study was to investigate the associations between early atherosclerosis, assessed by measurement of the common carotid artery IMT and microvascular diabetic complications, hypertension, hyperlipidaemia and smoking, in young patients with Type 1 DM.

Patients and Methods

Patients

We investigated prospectively 310 patients with Type 1 DM who attended our hospital as in- or outpatients. The inclusion criteria were age $\leq$ 40 years and diabetes duration of $\geq$ 2 years. All patients were treated with at least four daily insulin injections. Forty healthy volunteers of the same age group with no history of diabetes,

Abbreviations: AER albumin excretion rate, DM diabetes mellitus, IMT intima-media thickness

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hyperlipidaemia, hypertension or cardiovascular disease, most of them clinic personnel, were taken as control group 1. Control group 2 comprised 40 patients with recent onset of Type 1 DM (< 1 year before the examination). In these subjects, the measurement of lipids and albumin excretion rate (AER) was carried out when the patients had been on insulin for at least 2 weeks and had achieved good metabolic control. The clinical and biochemical characteristics of all the subjects are given in Table 1. All subjects gave their informed consent for the sonographic examination of the carotid arteries. The study was performed according to the principles of the declaration of Helsinki and was approved by the regional ethical committee (Baden-Wuerttemberg Board of Physicians).

Clinical and Biochemical Examination

Total plasma cholesterol and triglycerides were determined by standard enzymatic tests (Dimension, DADE, Munich, Germany) in a blood sample collected after an overnight fast (interassay imprecision for cholesterol VC 1.4–3.2 %, for triglycerides VC 1.8–3.2 %). HDL- and LDL- cholesterol were measured only in subjects with a total cholesterol > 5.2 mmol l^{-1} and therefore not included in the analysis.

The AER and the total protein were measured by nephelometry (Nephelometer BN II, Behring, Marburg, Germany) either in a 24-h urine collection in inpatients or in a 12-h overnight collection in outpatients. Nephropathy was defined according to Mogensen *et al.*,[30] as stage III: microalbuminuria (AER > 20 μg min^{-1} in at least two urine collections), or stage IV: overt proteinuria (> 500 mg $24\ h^{-1}$). Haemoglobin A_{1c} (HbA_{1c}) was mesaured by HPLC (Bio-Rad Diamat, Munich, Germany, non-diabetic range 4.3–6.1 %). These biochemical examinations were performed routinely for clinical purposes and were not done in healthy subjects. All laboratory tests were carried out by the Institute for Clinical Chemistry and Laboratory Medicine of the Buergerhospital Stuttgart with regular participation in internal and external quality control programmes (German legal administration).

Systolic and diastolic resting blood pressure was measured with a standard cuff sphygmomanometer before ultrasound examination with the subject seated. Patients were considered to be hypertensive asccording to Doria *et al*,[31] when in repeated measurements on different days the systolic blood pressure was > 140 mmHg or the diastolic blood pressure was > 90 mmHg, or if they were prescribed antihypertensive drug therapy. Retinopathy was determined as absent or present in any form by standardized fundus examination through dilated pupils by the ophthalmologist.

Sonographic Examination

Sonographically, the arterial wall can be seen as a complex of three layers. Next to the lumen, an echogenic

Table 1. Clinical and biochemical characteristics of control subjects and Type 1 DM patients

Group	Control 1 (healthy volunteers)	Control 2 (newly diagnosed patients)	Patients
n	40	40	310
Gender (M/F)	37.5/62.5	60/40	43.2/56.8
Age (years)	26.4 ± 4.6 (26.0; 16–35)	23.7 ± 6.5 (22.5; 14–39)	27.9 ± 6.5 (28.0; 14–40)
Smokers	23.7	35.0	37.7
Duration of diabetes (years)	–	(< 1)	11.7 ± 7.6 (11.0; 2–37)
HbA_{1c} (%)	–	10.3 ± 2.8 (10.3; 4.7–16.3)	8.8 ± 2.4 (8.3; 4.6–19.5)
Albumin excretion rate[a] (μg min^{-1})	–	5.5 (3.8, 7.9)	16.0 (12.0, 21.4)
Total cholesterol (mmol l^{-1})	–	4.5 ± 0.9 (4.6; 2.8–6.1)	5.1 ± 1.3 (4.9; 2.9–10.1)
Triglycerides (mmol l^{-1})	–	1.3 ± 0.9 (1.0; 0.6–4.4)	1.3 ± 0.8 (1.0; 0.4–5.8)
Systolic blood pressure (mmHg)	128.3 ± 12.8 (130.0; 105–150)	112.3 ± 11.2 (110.0; 80–130)	119.0 ± 15.5 (120.0; 90–155)
Diastolic blood pressure (mmHg)	77.5 ± 10.8 (80.0; 55–90)	67.8 ± 10.3 (70.0; 50–90)	74.3 ± 11.1 (75.0; 50–105)
Hypertension	0	0	12.3
Antihypertensive treatment	0	0	10.6
Nephropathy (AER > 20 μg min^{-1})	–	2.5	21.9
Retinopathy (any)	–	0	25.2

Data are means ± SD (median; range) or % except [a]AER: geometric mean (95 % CI).

line represents the lumen–intima interface, followed by a small zone of low echogenicity, which is part of the media and then a second and broader echogenic zone representing the media–adventitia interface.

We examined supine subjects, using an Ultramark 9 HDI sonographic system (ATL, Bothel, WA, USA) with a linear array wide frequency probe (L 10-5 38 mm, 5–10 MHz) with an axial resolution of 0.3 mm. The extracranial parts of the carotid arteries (right and left) were scanned cross-sectionally and longitudinally. We took an end diastolic frozen B-mode picture for measurement from the distal part of the common carotid artery which allows the best imaging of the wall layers.[9] We measured the IMT in a section about 1 to 1.5 cm proximal of the carotid bulb by setting two pairs of callipers manually at sites of greatest thickness from the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface. For evaluation, we included the maximum IMT (= greatest wall thickness from right or left side) of each patient. In other parts of the carotid artery (bulb, internal branch) the intima–media complex often is not so well represented and the measurement of the IMT is uncertain.[6,7,32] For methodological reasons,[5,9,32] we took measurements from the far wall only.

To assess the reproducibility of the IMT measurement, a total of 20 subjects were examined by two independent observers twice on the same occasion (interobserver variability) and an additional measurement was carried out by one observer on a second occasion, separated by 7 to 14 days (intraobserver variability). The interobserver correlation was 97 % and the intraobserver correlation 98 %. The mean absolute differences were 0.04 ± 0.06 mm and 0.03 ± 0.06 mm, respectively, and the coefficient of variation was 6.25 % for both variabilities. All measurements were performed by one investigator who was blinded at the time of the reading to the patient's complication status.

Statistical Analyses

Continuous variables are given as means ± standard deviation, median and range. As the AER was not normally distributed, the values were log-transformed for analysis and summarized in Tables 1 and 2 as the geometric mean with 95 % CI. Intergroup comparisons were made with the two-sided Student's *t*-test for independent samples or with nonparametrical tests (Mann–Whitney), where appropriate. A p value (two-sided) < 0.05 was considered statistically significant.

Clinical and biochemical parameters (age, gender, duration of diabetes, systolic and diastolic blood pressure, HbA_{1c}, AER, total cholesterol, triglycerides), microvascular diabetic complications (nephropathy, retinopathy), hypertension, and the smoking status were correlated with the common carotid artery IMT, and Pearson's correlation coefficients were calculated; the partial correlation coefficients when controlling for age and gender were also calculated.

A multiple linear regression analysis was performed to evaluate the effects of relevant parameters on the IMT of the Type 1 DM patients. Age and gender were forced into the model as confounders, then the other variables with presumably relevant correlation ($p \leq 0.10$) were selected from the univariate analysis and were sequentially entered into the model until no remaining candidate variable had a significance level of ≤ 0.10. SPSS for Windows 7.5 (SPSS Munich, Germany) was used for all statistical analyses.

Results

Mean maximum IMT was 0.52 ± 0.06 mm in healthy subjects (control group 1) and 0.50 ± 0.05 mm in newly diagnosed patients (control group 2) with a mean difference of 0.02 mm (95 % CI: –0.01, 0.04 mm) between both control groups, not significantly different. In the combined control groups, there were no significant differences in IMT between right and left carotid artery, men and women or smokers and non-smokers (≤ 0.01 mm within all subgroups) and no significant correlations between IMT and age or blood pressure.

In the group of patients with established (≥ 2 years duration) Type 1 DM, mean maximum IMT was 0.57 ± 0.13 mm, significantly greater than in both control groups ($p < 0.001$). The mean age of these patients was not significantly different from that of control group 1 (healthy subjects), although significantly higher than in control group 2 (Table 1).

When the patients were divided into subgroups with and without microvascular complications, the IMT was 0.55 ± 0.10 mm for patients without and 0.63 ± 0.17 mm for those with complications ($p < 0.001$). All other parameters besides gender and smoking status were also significantly different (Table 2).

The univariate analysis showed no correlation between IMT and smoking status or systolic blood pressure (Table 3). All other parameters were entered into the multiple linear regression analysis (Table 4). In the established DM group, besides age and gender, only triglycerides and nephropathy status were independently associated with an increase in IMT. Every 10 years of age contributed to an intima-media thickening of about 0.06 mm, male gender added about 0.03 mm. In patients without complications, none of the variables reached a significance level of ≤ 0.10 in the multivariate analysis.

Figures 1(a) and 1(b) show the correlations between age and IMT in patients with and without complications. This correlation was weaker in the latter. Figures 2(a) and 2(b) illustrate that there was no significant relationship between the duration of diabetes and IMT, in patients with or without complications. The correlation between IMT and log-transformed AER was weak (Figure 3), but IMT increased clearly with progressing nephropathy: 0.55 ± 0.12, 0.62 ± 0.10 and 0.66 ± 0.19 mm in patients

Table 2. Characteristics of patients with and without microvascular diabetic complications (nephropathy and/or retinopathy)

	Without complications	With complications	*p* value
n	211	99	–
Age (years)	26.8 ± 6.5	30.1 ± 6.0	< 0.001
Gender (M/F)	41.2/58.8	47.5/52.5	0.302
Duration of diabetes (years)	9.0 ± 6.0	17.4 ± 7.6	< 0.001
HbA_{1c} (%)	8.5 ± 2.4	9.3 ± 2.3	0.012
Smokers	36.5	40.4	0.509
Albumin excretion rate[a] (μg min^{-1})	4.6 (4.2, 5.2)	122.1 (75.5, 197.4)	< 0.001
Total cholesterol (mmol l^{-1})	4.8 ± 1.0	5.6 ± 1.5	< 0.001
Triglycerides (mmol l^{-1})	1.1 ± 0.7	1.5 ± 1.0	0.005
Systolic blood pressure (mmHg)	113.1 ± 13.6	125.5 ± 14.9	< 0.001
Diastolic blood pressure (mmHg)	70.1 ± 9.9	78.8 ± 10.7	< 0.001
Hypertension	1.4	35.4	< 0.001
Antihypertensive treatment	1	31.3	< 0.001

Data are means ± SD (median; range) or % except [a]AER: geometric mean (95 % CI).

without nephropathy, with albuminuria and with proteinuria (Figure 4 shows the means and 95 % CI of IMT in these subgroups).

Discussion

The measurement of the IMT of the carotid artery has been described by several authors using a variety of methods.[6,9,13,14,20,27,33–35] Although the absence of standardization of the IMT measurement makes it difficult to compare absolute values between different studies, good reproducibility within single studies can be achieved when well-trained investigators use the same equipment and the same standardized methods[1,9,13,14,32–34,36] and a recent study showed that the results were the same whether the maximum or the mean IMT were taken for analysis.[26]

IMT correlates with age.[1,2,20,33,37] In adults, increasing IMT can be observed after the 35th to 40th year of life.[13,38] We investigated only patients aged up to 40 years. In these subjects the influence of age is negligible, as confirmed in our healthy and diabetic control subjects and in our patients without complications. We found no effect of gender on the IMT in non-diabetic subjects and Type 1 DM patients without complications.

In young Type 1 DM patients with complications, the situation was different: their carotid artery IMT was clearly related to age and there was also a weak correlation to gender. The finding that in these patients the IMT was greater in males is in accordance with earlier observations of an unexplained male preponderance for the development of severe microvascular diabetic complications.[28,39]

Only a few studies have been performed on intima-media thickening in patients with Type 1 DM. All of them describe increased IMT in diabetic patients, compared to control subjects.[26] However, the variables which were found to be associated, differ. In our patients, diabetes duration was not related to the IMT, but microvascular complications had an association. Our results agree in part with those of Yokoyama *et al.*,[23] who found a significant relationship between carotid

Table 3. Univariate correlation analysis between possible predictor variables and the IMT in 310 Type 1 DM patients

Variable	Univariate/partial correlation coefficient	*p* value
Age (years)	0.351	< 0.001
Gender (0 = women, 1 = men)	0.175	< 0.01
Smokers (0 = no, 1 = yes)	0.026	0.649
Duration of diabetes (years)	0.141	< 0.05
HbA_{1c} (%)	0.101	0.078
Log albumin excretion rate (μg min^{-1})	0.135	< 0.05
Nephropathy (0 = no, 1 = microalbuminuria, 2 = proteinuria)	0.220	< 0.01
Systolic blood pressure (mmHg)	0.022	0.796
Diastolic blood pressure (mmHg)	0.174	< 0.05
Hypertension (0 = no, 1 = yes)	0.160	< 0.05
Total cholesterol (mmol l^{-1})	0.166	< 0.05
Triglycerides (mmol l^{-1})	0.265	< 0.001
Retinopathy (0 = no, 1 = yes)	0.184	< 0.01

Data are Pearson's correlation coefficients (age, gender) or partial correlation coefficients when controlling for age and gender (other variables).

Table 4. Multiple linear regression analysis of variables for the prediction of the IMT in 310 Type 1 DM patients

Variable	Partial regression coefficient	Standard error of regression coefficient	*p* value for regression coefficient
Age (years)	0.006	0.001	< 0.001
Gender (0 = women, 1 = men)	0.026	0.014	0.053
Nephropathy (0 = no, 1 = microalbuminuria, 2 = proteinuria)	0.033	0.010	0.002
Triglycerides (mmol l^{-1})	0.035	0.010	< 0.001

For each unit increase of the predictor variable, the regression coefficient reflects the mean increase of IMT.

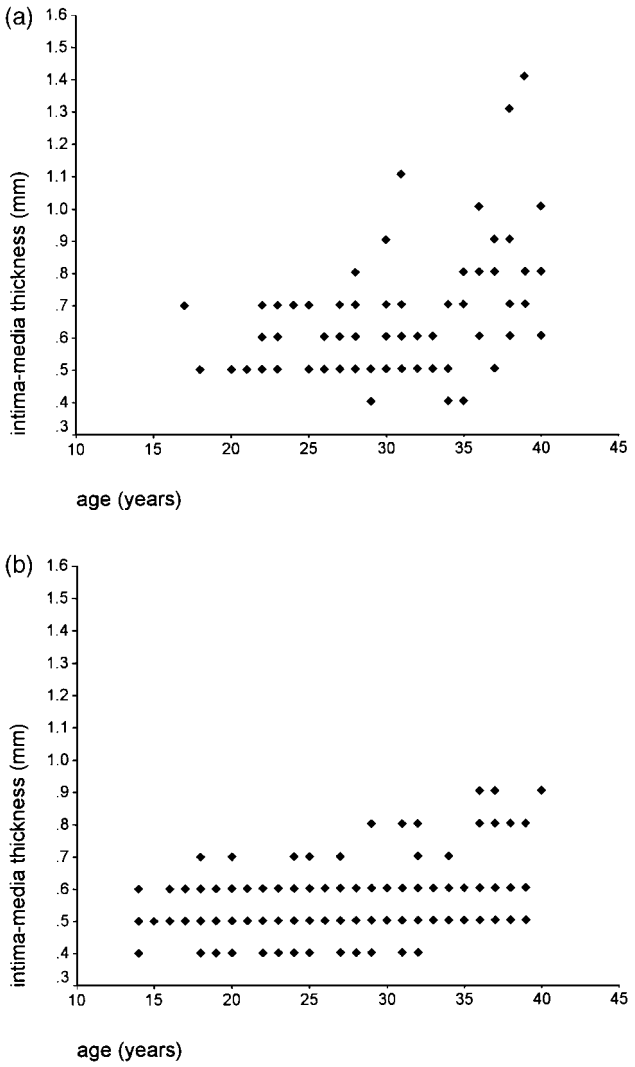

Figure 1. Correlation between age and IMT in (a) 99 Type 1 DM patients with microvascular complications (correlation coefficient r = 0.349) and (b) 211 Type 1 DM patients without macrovascular complications (correlation coefficient $r = 0.297$). Squares may represent more than one patient if the values of several subjects were coincidental

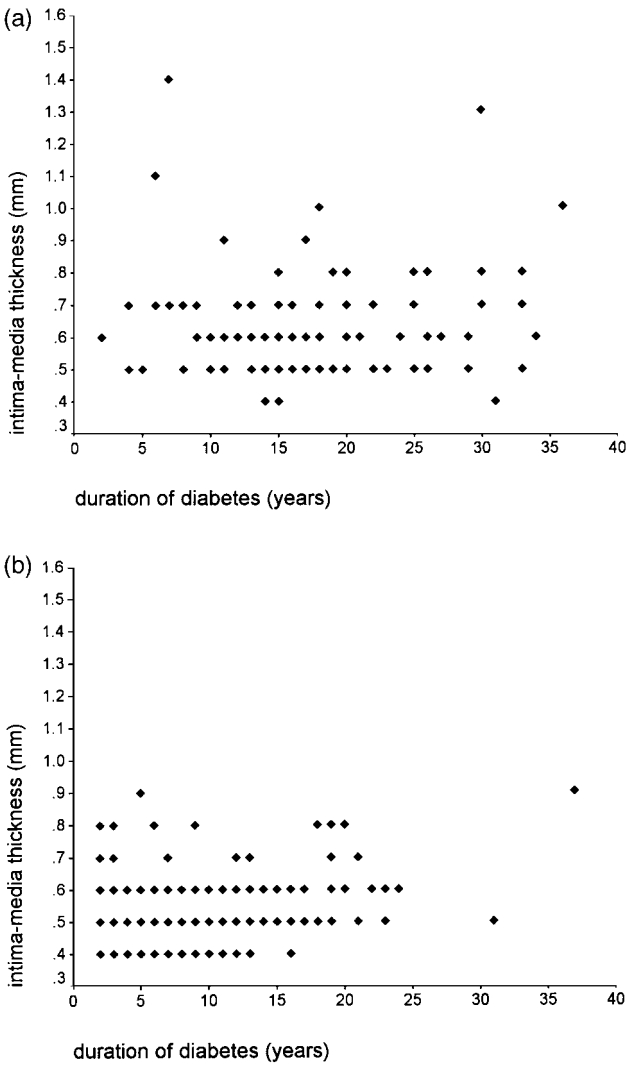

Figure 2. Correlation between duration of diabetes and IMT in (a) 99 Type 1 DM patients with microvascular complications (correlation coefficient r = 0.044) and (b) 211 Type 1 DM patients without microvascular complications (correlation coefficient $r = 0.105$). Squares may represent more than one patient if the values of several subjects were coincidental

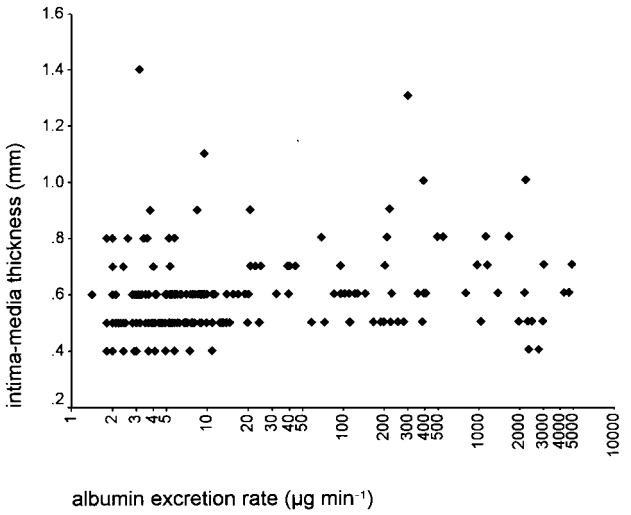

Figure 3. Correlation between albumin excretion rate (log-transformed scale) and IMT in 310 Type 1 DM patients

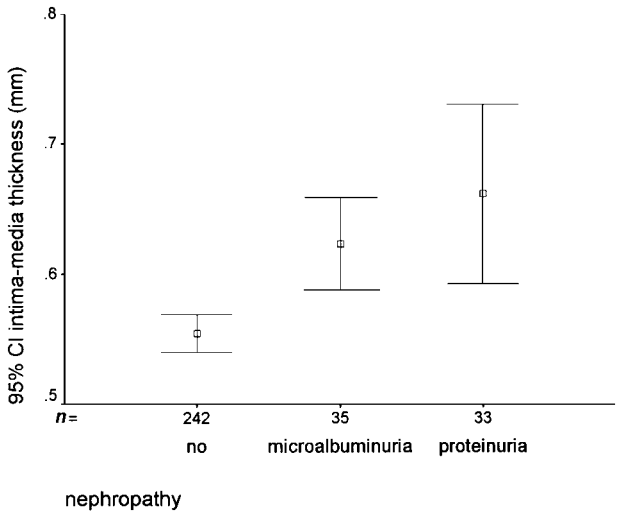

Figure 4. Mean IMT and 95 % CI according to nephropathy stages in 310 Type 1 DM patients

artery IMT and proliferative retinopathy, but not with the diabetes duration, in Type 1 DM patients of comparable age (but without proteinuria). In contrast, Yamasaki *et al.*[25] described a correlation between the IMT and the diabetes duration in very young patients, mostly children and adolescents. This study did not take diabetic complications into account. Kanters *et al.*[26] found age over 50 years and HbA_{1c} to be the only predictors for the carotid artery IMT in hyperlipidaemic Type 1 DM patients. In our patients, the HbA_{1c} was higher in patients with complications, but it did not contribute to the IMT in the multivariate analysis.

Previous studies in Type 1 DM patients have been smaller (12 to 105 subjects) than ours which may explain the partly different results. Our study of 310 subjects shows a strong association between nephropathy status and carotid artery wall thickening in young Type 1 DM patients, whereas we found only a weak correlation between AER and IMT. The carotid artery IMT increased with progressing nephropathy stage, i.e. microalbuminuria or overt proteinuria. These results agree with earlier observations that albuminuria is associated not only with microangiopathy in other regions (especially proliferative retinopathy), but also with widespread macrovascular lesions.[28,29,40,41] Nephropathy and the development of coronary heart disease have been correlated before in Type 1 DM[24,28,29,40,42] and recent studies in Type 2 DM also show an association between albuminuria and carotid artery IMT.[43,44]

Besides nephropathy, only the triglycerides contributed to the IMT in the multivariate analysis. This has been described before.[3,8] It is still unclear whether the triglycerides play a role as an independent risk factor for the development of atherosclerosis.[45] A known inverse correlation between triglycerides and HDL-cholesterol could explain our results but we did not have sufficient data to examine HDL-cholesterol. An association between triglycerides and AER was recently described in male Type 2 DM patients.[42]

In conclusion, our present study suggests that nephropathy is the main diabetes specific risk for intima–media thickening in young Type 1 DM patients. As the duration of diabetes does not influence the IMT, the presence of uncomplicated Type 1 DM alone does not seem to be associated with carotid artery wall thickening.

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